METHODS FOR PREPARING THERAPEUTICALLY ACTIVE CELLS USING MICROFLUIDICS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. Ser. No. 16/662,033, filed on Oct. 24, 2019, which is a continuation of U.S. Ser. No. 16/108,365, filed on Aug. 22, 2018, which issued as U.S. Pat. No. 10,844,353 on Nov. 24, 2020 and which claims the benefit of U.S. Provisional Patent Application No. 62/553,723, filed on Sep. 1, 2017; the benefit of U.S. Provisional Patent Application No. 62/567,553, filed on Oct. 3, 2017; the benefit of Provisional Patent Application No. 62/635,304, filed on Feb. 26, 2018; and the benefit of Provisional Patent Application No. 62/656,939, filed on Apr. 12, 2018; and, in addition, the is a continuation-in-part of PCT/US2017/057876, filed on Oct. 23, 2017. These prior applications are all incorporated by reference herein in their entireties.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant No. CA174121 and No. HL110574 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention is directed primarily to methods of preparing cells and compositions for therapeutic uses. The methods employ microfluidic devices that separate cells based on size.

BACKGROUND OF THE INVENTION

[0004] Cell therapy, and especially CAR-T cell therapy, has demonstrated extraordinary efficacy in treating B-cell diseases such as B-acute lymphoid leukemia (B-ALL) and B-Cell Lymphomas. As a result, the demand for autologous therapies has increased dramatically and development efforts have broadened to focus on cancers characterized by solid tumors, such as glioblastomas (Vonderheide, et al., *Immunol. Rev.* 257:7-13 (2014); Fousek, et al., *Clin. Cancer Res.* 21:3384-3392 (2015); Wang, et al., *Mol. Ther. Oncolytics* 3:16015 (2016); Sadelain, et al., *Nature* 545:423-431 (2017)). Targeted gene editing with CRISPR/Cas-9 in focused populations of autologous cells, such as stem cells, may further fuel demand (Johnson, et al., *Cancer Cell Res.* 27:38-58 (2017)).

[0005] The preparation of cells for personalized therapy is usually a labor-intensive process that relies on procedures adapted from blood banking or protein bioprocessing procedures which are poorly suited for therapeutic applications. Cell losses associated with processing steps are typically substantial (Hokland, et al., *Scand. J. Immunol.* 11:353-356 (1980); Stroncek, et al., *J. Transl. Med.* 12:241 (2014)), in part because of processes that use preparations that achieve cell specific separations (Powell, et al., *Cytotherapy* 11:923-935 (2009); TerumoBCT. ELUTRA Cell Separation System. Manufacturer recommendations for the Enrichment of Lymphocytes from Apheresis Residues) but do so at the expense of cell viability and yield (Chiche-Lapierre, Cytotherapy 18(6):547 (2016)). Thus, there is a need for more efficient processes.

SUMMARY OF THE INVENTION

[0006] The present invention is directed, inter alia, to methods of collecting and rapidly processing cells, particularly cells that have therapeutic uses. Many of the methods rely on Deterministic Lateral Displacement (DLD), a process that involves flowing a sample through a microfluidic device containing a specifically designed array of microposts that are tilted at a small angle from the direction of fluid flow (Davis, et al., *Proc. Natl. Acad. Sci. USA* 103:14779-14784 (2006); Inglis, et al., *Lab Chip* 6:655-658 (2006); Chen, et al., *Biomicrofluidics.* 9(5):054105 (2015)). Cells larger than the target size of the micropost array may be gently deflected ("bumped") by the microposts into a stream of clean buffer, effectively separating them from smaller, non-deflected cells and particles, while simultaneously washing the cells in a process that is non-injurious. Advantageous characteristics of DLD with respect to cell processing are described in Table 1:

TABLE 1

Intrinsic Properties of DLD and Their Implications for Cell Processing

DLD Feature	Enablement	Implications
Uniform feature and gap size	Fractionate complex mixtures based on size with ability to discriminate particles to within ~0.5 μm.	Uniform and gentle de-bulking of platelet and RBC from blood products without centrifugation up to 99.99% efficiency Eliminates open solutions such as Ficoll, and avoids need for harsh hypertonic solutions (Elutriation).
	Ability to mix different Dc within the same device	Use of sequential cut-offs to manage highly heterogeneous fractionations
	Cell Washing & Buffer Exchange	Cell Washing >99.9% removal in single pass Potential to improve and remove cell culture while maintaining closed system ensuring viable cells.
	Concentration	Concentration of cells in culture to make downstream processing seamless. Minimize reagent expense without requiring open centrifugation or transfer losses.